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# Polyphasic characterization and GC-MS metabolite profiling of a *Streptomyces* isolate affiliated with *Streptomyces hygroscopicus* from pineapple rhizosphere in Lampung, Indonesia

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**Abstract.** *Aeny TN, Helina S, Sudarsono H, Dewi NKES, Dirmawati SR. 2026. Polyphasic characterization and GC-MS metabolite profiling of a* Streptomyces *isolate affiliated with* Streptomyces hygroscopicus *from pineapple rhizosphere in Lampung, Indonesia. Biodiversitas 27 (3): d270340. https://doi.org/10.13057/biodiv/d270340*. Members of the genus *Streptomyces* represent taxonomically diverse and metabolically versatile actinobacteria commonly associated with rhizosphere ecosystems. This study presents a polyphasic characterization of a *Streptomyces* isolate obtained from the pineapple (*Ananas comosus*) rhizosphere in Lampung, Indonesia, integrating morphological, molecular, and metabolite profiling approaches. Morphological examination using scanning electron microscopy revealed extensively branched vegetative hyphae and rectiflexibiles-type spore chains with smooth surfaces, consistent with diagnostic features of the genus. Molecular identification based on 16S rRNA gene sequencing (~1,450 bp) placed the isolate within the *Streptomyces hygroscopicus* clade (GenBank accession no. PZ049818), showing low genetic divergence (<0.02 substitutions per site) relative to closely related strains, although species-level resolution remains limited using 16S rRNA alone. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of ethyl acetate extracts detected 53 volatile and semi-volatile compounds, with similarity indices ranging from 66% to 96%; compounds with similarity indices ≥80% were considered reliable identifications. Major constituents included 3-deoxy-D-mannoic lactone (6.92%), (S)-(+)-2-amino-3-methyl-1-butanol (6.42%), 2-methylbutanoic anhydride (5.74%), catechol (5.16%), and 9,12-octadecadienoic acid (Z,Z) (4.58%), representing diverse chemical classes such as organic acids, alcohols, phenolics, and fatty acids. Although limited to a single isolate and qualitative metabolite profiling, this study provides baseline taxonomic and metabolomic data for pineapple-associated *Streptomyces*, serving as a foundation for future genomic, functional, and biocontrol-related studies in tropical agroecosystems.

**Keywords:** Actinomycetes, GC-MS analysis, pineapple rhizosphere, secondary metabolites, *Streptomyces hygroscopicus*

## INTRODUCTION

The genus *Streptomyces* is one of the most diverse and metabolically versatile groups within the phylum Actinobacteria. These Gram-positive bacteria are ubiquitous in soil and rhizosphere ecosystems, where they drive organic matter decomposition, nutrient cycling, and microbial community regulation. They are morphologically distinguished by branching filamentous hyphae, aerial mycelia, and complex spore chains (Kämpfer et al. 2014; Behie et al. 2017; Aeny et al. 2018; Hashim et al. 2026), exhibiting ecological adaptability through complex developmental cycles and extracellular enzymes capable of degrading polymers such as cellulose and chitin (Barka et al. 2016; Chater 2016). These bacteria are also renowned for producing over two-thirds of commercially available antibiotics via numerous biosynthetic gene clusters encoding polyketides, non-ribosomal peptides, terpenoids, and other specialized metabolites (Doroghazi and Metcalf 2013; Selim et al. 2021). Yet, many clusters remain inactive under laboratory conditions, highlighting untapped metabolic potential (Rutledge and Challis 2015). Although actinomycetes from various cropping systems have been studied for antimicrobial and plant growth-promoting properties (Torres-Rodríguez et al. 2022; Khan et al. 2023b), their diversity and functional roles in specific rhizosphere environments, such as those of tropical fruits, remain underexplored.

Numerous studies have established the efficacy of *Streptomyces* species in suppressing fungal and bacterial pathogens through antibiosis, resource competition, and the secretion of lytic enzymes and bioactive metabolites. This antagonistic potential has been demonstrated against diverse phytopathogens, including *Magnaporthe oryzae* (Law et al. 2017), *Gloeophyllum trabeum* (Jung et al. 2018), *Ralstonia solanacearum* (Kaari et al. 2022), and *Fusarium* spp. in cereals (Vuong et al. 2025). Within the rhizosphere, a dynamic niche governed by root exudates, these actinobacteria facilitate plant growth through the synthesis of phytohormones and siderophores, phosphate solubilization, and the induction of systemic resistance (Philippot et al. 2013; Viaene et al. 2016). Their filamentous morphology further contributes to this function by ensuring robust root colonization. Despite their dual role as potent biocontrol agents and biofertilizers, the diversity and function of rhizosphere-associated *Streptomyces* in pineapple

(*Ananas comosus* (L.) Merr.) cultivation remain under-researched. Given that pineapple is a vital economic commodity, particularly in Lampung, Indonesia, addressing this knowledge gap is imperative. Understanding these microbial communities is a critical step toward harnessing their biotechnological potential to enhance pineapple productivity and ensure the sustainability of local agroecosystems.

Comprehensive characterization of *Streptomyces* requires polyphasic taxonomic approach integrating morphological, molecular, and chemotaxonomic data. Morphological assessment of colony pigmentation, mycelial architecture, and spore ornamentation provides initial identification, refined by techniques such as Scanning Electron Microscopy (SEM) for observing hyphal and spore ultrastructure (Shrivastava et al. 2015). However, morphological plasticity within the genus necessitates molecular confirmation. Molecular identification using 16S rRNA gene sequencing enables phylogenetic placement through comparison against reference sequences in databases such as NCBI GenBank and EzBioCloud (Yoon et al. 2017). Although widely accepted, 16S rRNA analysis may not fully resolve closely related species due to high sequence conservation (Kim et al. 2014), underscoring the need for complementary analytical approaches. Metabolite profiling using Gas Chromatography-Mass Spectrometry (GC-MS) detects volatile and semi-volatile secondary metabolites and generates strain-specific chemical fingerprints, providing additional insights into strain-level diversity and metabolic expression (Schöller et al. 2002; Ifediora et al. 2023).

This study aims to characterize a *Streptomyces* isolate obtained from pineapple rhizospheres in Lampung, Indonesia, with particular emphasis on metabolomic characterization, using an integrated approach combining morphological examination, 16S rRNA-based phylogenetic analysis, and GC-MS metabolite profiling. We hypothesize that rhizosphere-derived isolates exhibit distinct morphological, phylogenetic, and metabolite profiles that may reflect their ecological adaptation and potential functional roles. Accordingly, the objectives of this study are to: (i) characterize the isolate using a polyphasic taxonomic approach, (ii) determine its phylogenetic affiliation based on 16S rRNA gene analysis, and (iii) profile its volatile and semi-volatile metabolites using GC-MS to provide baseline insights into its biochemical potential. This study contributes fundamental information on actinomycete diversity in pineapple agroecosystems and provides a foundation for future ecological and biotechnological investigations.

## MATERIALS AND METHODS

### Exploration of *Streptomyces* from the pineapple rhizosphere

Rhizosphere soil samples were collected from a pineapple plantation located at PT Great Giant Foods (PT GGF), Central Lampung District, Lampung Province, Indonesia (approximately 4°52′S, 105°16′E). The soil in the study area is predominantly Ultisol, characterized by strongly acidic pH (3.9-6.2; mean ~4.3-4.4), typical of tropical pineapple-growing regions in Lampung. Detailed soil physicochemical parameters (e.g., organic carbon, moisture, and nutrient levels) were not measured in this study. Sampling was conducted from five healthy, actively growing pineapple (*A. comosus*) plants randomly selected within the plantation area. The sampled plants were at the vegetative growth stage, approximately 6-8 months after planting, prior to floral induction, when root development and rhizosphere microbial activity are typically well established.

Rhizosphere soil was collected by carefully uprooting the plants and gently shaking off loosely attached soil. The soil remaining tightly adhering to the root surface (approximately 0-5 mm from the root zone) was considered rhizosphere soil and was aseptically collected using sterile spatulas. Sampling was conducted during the dry season under typical plantation management conditions.

Approximately 200-300 g of rhizosphere soil per sampling point was placed into sterile polyethylene bags, labeled, and transported to the Laboratory of Plant Diseases, Department of Plant Protection, Universitas Lampung, for further analysis. Samples were refrigerated at 4°C and processed within 24-48 hours after collection. For isolation of actinomycetes, 10 g of rhizosphere soil was suspended in 90 mL sterile distilled water and serially diluted up to $10^{-5}$. Aliquots (0.1 mL) of appropriate dilutions were spread onto starch casein agar supplemented with nystatin (50 μg $mL^{-1}$) to suppress fungal growth. Plates were incubated at 28°C for 7-10 days. Colonies showing typical *Streptomyces*-like morphology (powdery appearance with aerial mycelia) were subcultured repeatedly on ISP2 agar to obtain pure cultures.

### Morphological characterization and identification

Morphological characterization was conducted as part of the genus-level identification using a polyphasic taxonomic approach that integrates morphological, physiological, and molecular characteristics to improve taxonomic reliability and support accurate actinomycetes identification (Stackebrandt and Schumann 2006). Pure cultures were streaked onto glucose-yeast extract-malt extract (GYM) agar (ISP medium 5) and oatmeal agar (ISP medium 3) and incubated at 28°C for 7-14 days. Colony morphology, including aerial and substrate mycelium coloration, diffusible pigment production, colony texture (powdery/butyrous), and growth patterns (elevated/flat), was systematically documented under daylight and transmitted light. Spore mass color was classified according to the International Streptomyces Project (ISP) color chart (Shirling and Gottlieb 1966).

Microscopic features were examined using the slide culture technique on GYM agar blocks. Hyphal branching, aerial mycelium formation, and spore chain arrangements were observed at 1000× magnification under oil-immersion light microscopy (Dietz and Mathews 1969). Selected isolates were subjected to Scanning Electron Microscopy (SEM) analysis (Kumar et al. 2011), conducted at the Integrated Laboratory, Universitas Lampung, Indonesia. Aerial hyphae and spore surfaces were fixed in 2.5% glutaraldehyde, dehydrated in ethanol series (30-100%),

critical-point dried, gold-sputtered, and imaged at 10-15 kV to resolve surface ornamentation (smooth/warty/ rugose). Among the obtained isolates, one representative *Streptomyces* isolate exhibiting distinct colony morphology and stable growth characteristics was selected for further polyphasic characterization.

**Molecular identification and phylogenetic analysis**

Molecular identification was conducted at the Laboratory of Plant Diseases, Department of Plant Protection, Faculty of Agriculture, Universitas Lampung, Indonesia. The procedure followed the standard protocol described by Rante et al. (2024) with slight modifications. Genomic DNA was extracted from 7-10-day-old pure cultures grown on ISP2 agar using the Zymo Research Genomic DNA Extraction Kit (Zymo Genomic DNA Kit) according to the manufacturer's protocol. Briefly, bacterial biomass was harvested from the agar surface and subjected to cell lysis, DNA binding, washing, and elution steps following the kit instructions. DNA concentration and purity were assessed by agarose gel electrophoresis and spectrophotometric measurement.

Amplification of the 16S rRNA gene was performed using universal bacterial primers fD1 (5'-AGA GTT TGA TCC TGG CTC AG-3') and rP2 (5'-ACG GCT ACC TTG TTA CGA CTT-3'), which amplify an approximately 1,500 bp fragment (Marín et al. 2012). The assembled 16S rRNA gene sequence used for phylogenetic analysis was approximately 1,450 bp in length. PCR reactions were carried out in a total volume of 25 µL using RedMix Master Mix (SolGent, South Korea). Each reaction mixture consisted of 12.5 µL RedMix Master Mix, 1 µL primer fD1 (10 pmol), 1 µL primer rP2 (10 pmol), 1 µL template DNA, and 9.5 µL nuclease-free water.

PCR amplification was performed in a thermal cycler (Eppendorf) under the following conditions: initial denaturation at 95°C for 3 min; 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 50 s, and extension at 72°C for 1 min; followed by a final extension at 72°C for 7 min. PCR products were separated by electrophoresis on 1% agarose gel prepared in 1× TAE buffer and visualized under a UV transilluminator. Amplicons of the expected size (~1,500 bp) were purified and sent for sequencing to Genetika Science (Jakarta, Indonesia).

The obtained partial 16S rRNA gene sequences were edited and assembled using BioEdit software v7.2.5 (Hall 1999). Sequence similarity was determined by comparing the sequences against reference sequence databases using NCBI BLAST and the EzBioCloud server (Yoon et al. 2017). Phylogenetic analysis was performed using the neighbor-joining method implemented in MEGA 12 (Kumar et al. 2023). Sequences were aligned using the MUSCLE algorithm, and evolutionary distances were calculated using the Kimura 2-Parameter model. The robustness of the tree topology was evaluated using bootstrap analysis with 1,000 replicates.

**GC-MS analysis of *Streptomyces***

GC-MS analysis was performed to identify volatile and semi-volatile metabolites for chemical characterization. The analytical procedure followed protocols described by Mondal and Thomas (2022) and Cunha et al. (2025) with slight modifications. For metabolite production, the *Streptomyces* isolate was cultured in ISP2 broth and incubated at 28°C for 7 days under shaking conditions (150 rpm). Crude ethyl acetate extracts obtained from *Streptomyces* cultures were evaporated to dryness under reduced pressure and subsequently redissolved in methanol at a concentration of 1 mg $mL^{-1}$. The solution was filtered through a 0.22 µm PTFE syringe filter prior to injection. A medium blank control (uninoculated culture medium subjected to the same extraction procedure) was analyzed under identical GC-MS conditions to exclude background compounds originating from the culture medium or solvents.

GC-MS analysis was carried out using an Agilent 7890B gas chromatograph coupled with a 5977A mass selective detector equipped with an HP-5MS capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). The oven temperature was initially set at 70°C (held for 2 min), then increased to 300°C at a rate of 10°C $min^{-1}$ and held for 10 min. Helium was used as the carrier gas at a constant flow rate of 1.2 mL $min^{-1}$ in splitless injection mode. The injector temperature was maintained at 250°C, and the transfer line temperature was set at 280°C. Mass spectra were recorded in Electron Ionization (EI) mode at 70 eV over a mass range of m/z 50-550. The GC-MS analysis was conducted for qualitative metabolite profiling to characterize the chemical composition of the extract, rather than for quantitative comparison among biological replicates. Compound identification was performed by comparing the obtained spectra with the NIST 14 mass spectral library database. Similarity indices ≥80% were considered reliable identifications, whereas compounds with lower similarity values were reported as tentative assignments. Identification was further supported by comparison of retention indices with reference values when available. Relative peak areas were used for semi-quantitative estimation of metabolite abundance.

## RESULTS AND DISCUSSION

**Morphological characteristics of the *Streptomyces* isolate**

The isolate exhibited well-developed mycelia with extensively branched hyphae, consistent with typical *Streptomyces* morphology (Figure 1). Scanning Electron Microscopy (SEM) revealed aerial spore chains arranged in a rectiflexibiles pattern (hook-shaped to flexuous). Individual spores were spherical to oval in shape, measuring approximately 0.5-1 µm in diameter, with smooth surface ornamentation at 10,000× magnification. These diagnostic morphological features are consistent with those commonly reported for members of the genus *Streptomyces*.

**Phylogenetic analysis of *Streptomyces hygroscopicus***

Molecular identification based on 16S rRNA gene sequences unequivocally assigned the isolate from the pineapple rhizosphere to the genus *Streptomyces*. Preliminary BLAST analysis (https://blast.ncbi.nlm.nih.gov/) corroborated this taxonomic placement, and the obtained sequence was

subsequently deposited in the NCBI GenBank database under the accession number PZ049818.

Phylogenetic analysis based on the 16S rRNA gene sequence placed the isolate within the *S. hygroscopicus* clade (Figure 2). The isolate (GenBank accession no. PZ049818) clustered closely with reference strains of *S. hygroscopicus*, including *S. hygroscopicus* subsp. *hygroscopicus* NBRC 11206 and other related strains. However, species-level resolution remains limited when using 16S rRNA gene sequences alone. The phylogenetic tree showed that the isolate grouped within the *S. hygroscopicus* lineage and was clearly separated from other closely related *Streptomyces* species such as *S. violaceusniger*, *S. javensis*, and *S. sporoclivatus*. This clustering pattern indicates that the isolate is phylogenetically affiliated with *S. hygroscopicus*, consistent with the morphological characteristics observed for the isolate. However, given the limited resolution of 16S rRNA gene analysis within the genus *Streptomyces*, the isolate is conservatively referred to as affiliated with *S. hygroscopicus* rather than being assigned to a definitive species.

To further quantify the evolutionary divergence between the isolate and its phylogenetic neighbors, a pairwise genetic distance analysis was conducted based on 16S rRNA gene sequences and visualized via a comprehensive heatmap (Figure 3). The heatmap showed that most pairwise comparisons among *Streptomyces* strains were characterized by low genetic distance values, as indicated by the predominance of yellow to light-green coloration. Pairwise genetic distances among the closely related strains were generally below 0.02 substitutions per site, indicating high sequence similarity.

The pineapple rhizosphere isolate (PZ049818) exhibited very low genetic distances relative to several reference strains, particularly those within the same cluster, indicating strong genetic relatedness. No distinct high-distance separation (dark orange blocks) was observed between the isolate and its closest reference strains, suggesting minimal sequence divergence at the 16S rRNA locus.

A small number of comparisons showed moderately higher genetic distance values (orange shades), forming localized blocks in the matrix. However, the overall pattern demonstrates that the isolate falls within a genetically conserved cluster of *Streptomyces*, supporting its close taxonomic affiliation within the genus.

## GC-MS chromatogram profile of *Streptomyces* volatile and semi-volatile metabolites

GC-MS analysis of the *Streptomyces* extract detected a total of 53 compounds based on spectral matching with the NIST 14 library, with similarity indices ranging from 66% to 96% (Table 1). Following widely accepted GC-MS identification criteria, compounds with similarity indices ≥80% were treated as reliable identifications, whereas compounds with similarity values <80% were categorized as tentative matches. The compounds with the highest relative peak areas included 3-deoxy-D-mannoic lactone (6.92%), (S)-(+)-2-amino-3-methyl-1-butanol (6.42%), 2-methylbutanoic anhydride (5.74%), catechol (5.16%), 5-oxotetrahydrofuran-2-carboxylic acid (4.96%), and 9,12-octadecadienoic acid (Z,Z) (4.58%). These metabolites represent several chemical classes detected in the extract, including organic acids, alcohols, phenolic compounds, heterocyclic derivatives, and fatty acids.

The GC-MS profile reflects a diverse metabolite composition consisting of compounds associated with both primary and secondary metabolism. Several detected compounds correspond to primary metabolic products, particularly sugar derivatives involved in carbohydrate metabolism, including xylitol, 3-deoxy-D-mannoic lactone, D-glycero-D-galacto-heptose, and 3-deoxy-D-mannonic acid.

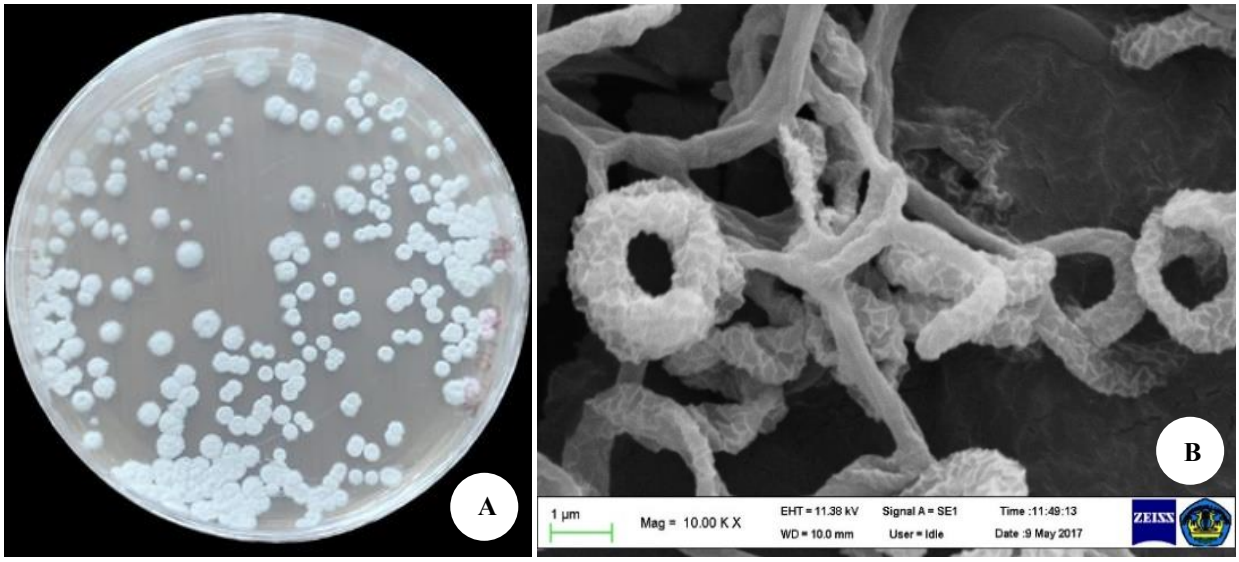


**Figure 1.** A. Morphological characteristics of *Streptomyces hygroscopicus*. Colony morphology on agar medium showing typical *Streptomyces* growth. B. Scanning Electron Micrograph (SEM) at 10,000× magnification showing extensively branched hyphae and aerial spore chains arranged in a rectiflexibiles (hook-shaped to flexuous) pattern

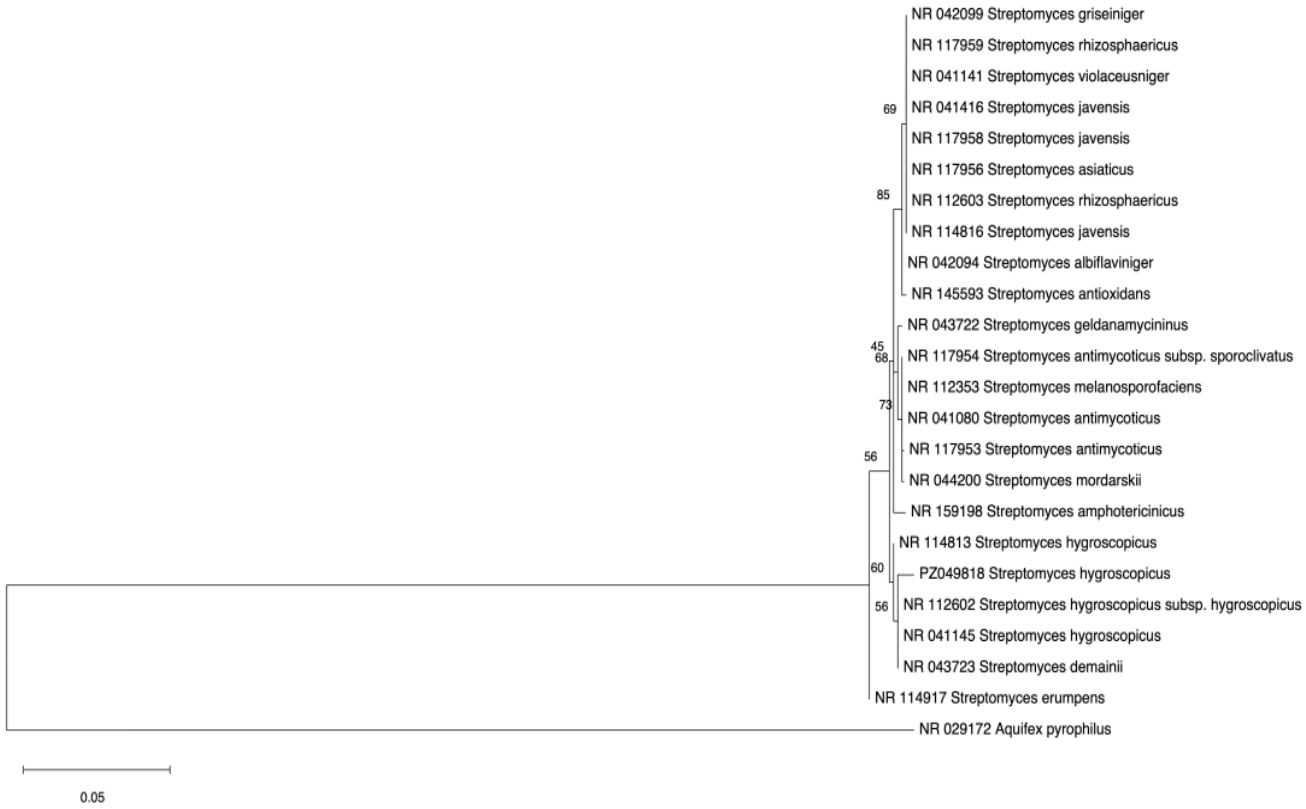


**Figure 2.** Phylogenetic tree based on 16S rRNA gene sequences showing the relationship between *Streptomyces hygroscopicus* isolate (GenBank accession no. PZ049818) and selected reference *Streptomyces* strains retrieved from GenBank

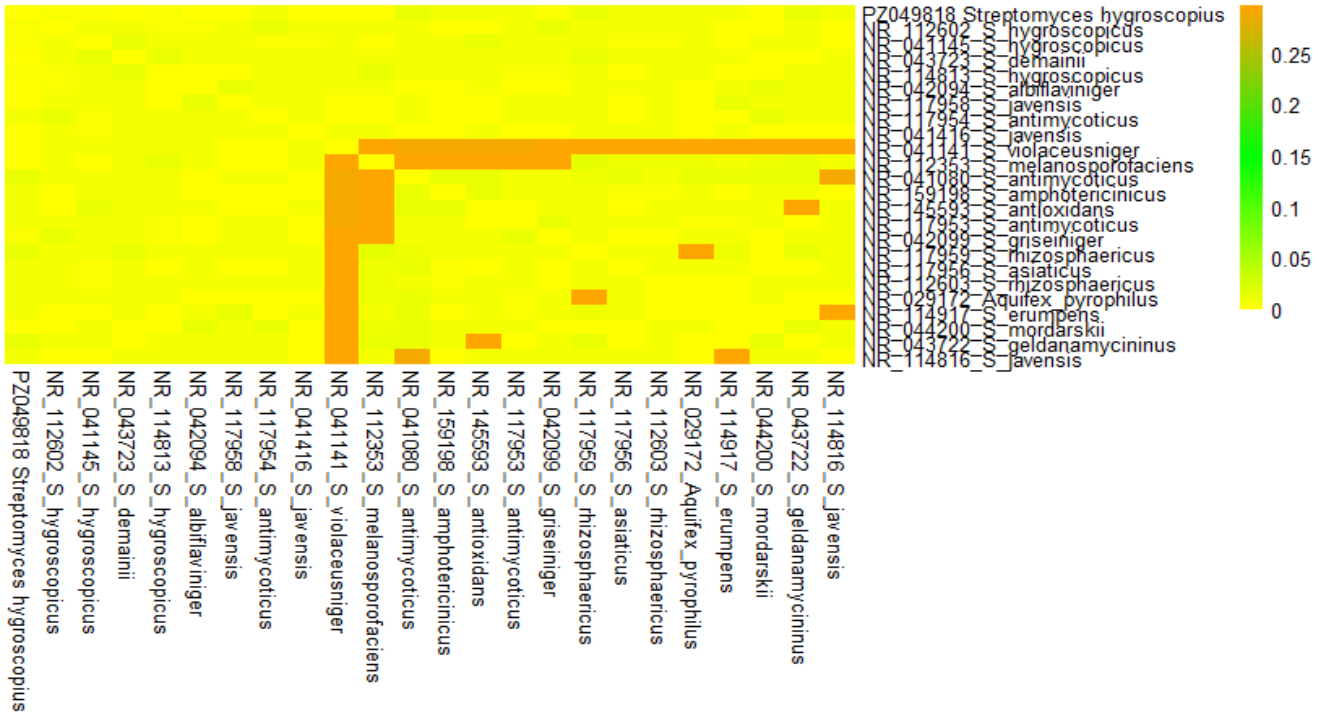


**Figure 3.** Heatmap of pairwise genetic distances based on 16S rRNA gene sequences between *Streptomyces hygroscopicus* isolates (GenBank accession no. PZ049818) and reference *Streptomyces* strains retrieved from GenBank

**Table 1.** GC-MS-identified volatile and semi-volatile compounds from *Streptomyces* isolate associated with pineapple rhizosphere. Compounds were categorized into major chemical classes (e.g., fatty acids, alcohols, phenolics, and heterocyclic compounds) to facilitate interpretation of metabolite composition. The m/z values represent the base peak (most abundant ion) in the mass spectrum.

| Peak no. | RT (min) | Area (%) | Similarity (%) | m/z | Compound name | Compound class | Identification level |
|---|---|---|---|---|---|---|---|
| 1 | 4.268 | 6.42 | 82 | 72.0 | (S)-(+)-2-Amino-3-methyl-1-butanol | Alcohol | Reliable |
| 2 | 4.499 | 0.43 | 84 | 60.0 | Propanoic acid, 3-hydroxy- | Organic acid | Reliable |
| 3 | 4.576 | 0.73 | 94 | 98.05 | 1,2-Cyclopentanedione | Ketone | Reliable |
| 4 | 5.213 | 0.64 | 93 | 101.05 | 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one | Heterocyclic (furanone) | Reliable |
| 5 | 5.257 | 0.62 | 86 | 74.05 | Diethanolamine | Amine (alkanolamine) | Reliable |
| 6 | 5.406 | 1.13 | 71 | 57.0 | Pentane, 1-bromo-3-methyl- | Halogenated hydrocarbon | Tentative |
| 7 | 5.912 | 0.39 | 91 | 114.05 | 3(2H)-Furanone, 4-hydroxy-5-methyl- | Heterocyclic (furanone) | Reliable |
| 8 | 6.037 | 0.37 | 76 | 102.05 | Butanedioic acid, monopropargyl ester | Ester (dicarboxylic acid derivative) | Tentative |
| 9 | 6.105 | 0.47 | 88 | 128.05 | 2,5-Dimethylfuran-3,4(2H,5H)-dione | Heterocyclic (furanone) | Reliable |
| 10 | 6.368 | 2.89 | 91 | 126.05 | Thymine | Nucleobase (pyrimidine) | Reliable |
| 11 | 6.45 | 0.85 | 82 | 55.0 | 1-Butene, 4-iodo- | Halogenated hydrocarbon | Reliable |
| 12 | 7.036 | 0.99 | 90 | 102.05 | Ethanamine, N-ethyl-N-nitroso- | Amine (nitroso compound) | Reliable |
| 13 | 7.145 | 3.69 | 94 | 144.0 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | Heterocyclic (pyranone) | Reliable |
| 14 | 7.25 | 1.15 | 77 | 74.05 | Butanoic acid, 4-chloro-, methyl ester | Ester (halogenated) | Tentative |
| 15 | 7.31 | 2.05 | 66 | 142.0 | 2-(tert-Butyldime-thylsilyl) thiazole | Heterocyclic (thiazole derivative) | Tentative |
| 16 | 7.466 | 3.92 | 85 | 84.0 | (S)-5-Hydroxymethyl-2[5H]-furanone | Heterocyclic (furanone) | Reliable |
| 17 | 7.608 | 5.74 | 85 | 85.05 | 2-Methylbutanoic anhydride | Anhydride (organic acid derivative) | Reliable |
| 18 | 7.657 | 5.16 | 77 | 61.0 | Catechol | Phenolic compound | Tentative |
| 19 | 7.898 | 4.96 | 93 | 85.0 | 5-Oxotetrahydrofuran-2-carboxylic acid | Heterocyclic (lactone) | Reliable |
| 20 | 7.965 | 2.12 | 93 | 97.05 | 5-Hydroxymethylfurfural | Aldehyde (furan derivative) | Reliable |
| 21 | 8.171 | 4.67 | 81 | 103.05 | 3-Acetoxy-3-hydroxypropionic acid, methyl ester | Ester (organic acid derivative) | Reliable |
| 22 | 8.297 | 0.65 | 71 | 61.0 | Xylitol | Sugar alcohol (polyol) | Tentative |
| 23 | 8.63 | 5.72 | 72 | 55.0 | 1-Propanol, 3-methoxy-2-(methoxymethyl)-2-methyl- | Alcohol (ether derivative) | Tentative |
| 24 | 8.857 | 0.53 | 77 | 57.0 | Undecane, 2-methyl- | Alkane (branched hydrocarbon) | Tentative |
| 25 | 9.075 | 0.46 | 83 | 73.0 | 2-t-Butyl-4-methyl-5-oxo-[1,3]dioxolane-4-carboxylic acid | Heterocyclic (dioxolane derivative) | Reliable |
| 26 | 9.21 | 1.4 | 82 | 103.05 | Butanedioic acid, 2-hydroxy-2-methyl-, (S)- | Organic acid | Reliable |
| 27 | 9.425 | 0.43 | 81 | 55.0 | Pentanoic acid, 2-hydroxy-, methyl ester | Ester (hydroxy acid) | Reliable |
| 28 | 9.527 | 1.65 | 87 | 126.05 | 1,2,3-Benzenetriol | Phenolic compound | Reliable |
| 29 | 9.612 | 0.58 | 71 | 142.0 | 2-Isobutoxy-5,5-dimethyl-[1,3,2]dioxaborinane | Organoboron compound | Tentative |
| 30 | 9.873 | 2.13 | 78 | 101.0 | Butanamide, 2-hydroxy-N,2,3,3-tetramethyl- | Amide | Tentative |
| 31 | 10.118 | 1.79 | 83 | 57.0 | 2-(Isobutoxymethyl) oxirane | Epoxide (oxirane derivative) | Reliable |
| 32 | 11.485 | 6.92 | 76 | 57.05 | 3-deoxy-D-mannoic lactone | Lactone (sugar acid derivative) | Tentative |
| 33 | 11.601 | 3.65 | 80 | 73.05 | D-glycero-D-galacto-heptose | Sugar (carbohydrate) | Reliable |
| 34 | 11.656 | 5.33 | 85 | 57.05 | 3-deoxyD-mannoic lactone | Lactone (sugar acid derivative) | Reliable |
| 35 | 11.82 | 1.41 | 68 | 137.05 | Oxirane, hexadecyl- | Epoxide (long-chain) | Tentative |
| 36 | 11.869 | 1.79 | 85 | 57.0 | 3-deoxy-D-mannonic acid | Organic acid (sugar acid) | Reliable |
| 37 | 12.017 | 0.5 | 67 | 84.0 | 2H-Pyran-2-acetic acid, tetrahydro- | Heterocyclic (pyran derivative) | Tentative |
| 38 | 12.134 | 0.43 | 92 | 73.05 | Tetradecanoic acid | Fatty acid (saturated) | Reliable |
| 39 | 12.401 | 0.21 | 94 | 73.05 | Tetradecanoic acid | Fatty acid (saturated) | Reliable |
| 40 | 12.858 | 0.33 | 91 | 73.05 | Pentadecanoic acid | Fatty acid (saturated) | Reliable |
| 41 | 12.924 | 1.44 | 84 | 57.05 | i-Propyl 12-methyltetradecanoate | Ester (fatty acid ester) | Reliable |
| 42 | 13.115 | 0.18 | 95 | 73.05 | Pentadecanoic acid | Fatty acid (saturated) | Reliable |
| 43 | 13.225 | 0.17 | 82 | 100.05 | N1-(2-Dimethylamino-ethyl)-3,N1,N2,N2-tetramethyl-butane-1,2-diamine | Amine (polyamine) | Reliable |
| 44 | 13.553 | 0.63 | 92 | 73.0 | n-Hexadecanoic acid | Fatty acid (saturated) | Reliable |
| 45 | 13.694 | 0.36 | 93 | 55.05 | Palmitoleic acid | Fatty acid (unsaturated) | Reliable |
| 46 | 13.807 | 2.44 | 96 | 73.0 | n-Hexadecanoic acid | Fatty acid (saturated) | Reliable |
| 47 | 14.228 | 0.11 | 86 | 57.05 | Pentadecanoic acid | Fatty acid (saturated) | Reliable |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 48 | 14.287 | 0.24 | 84 | 57.05 | i-Propyl 14-methylhexadecanoate | Ester (fatty acid ester) | Reliable |
| 49 | 14.408 | 0.39 | 94 | 55.05 | cis-10-Heptadecenoic acid | Fatty acid (unsaturated) | Reliable |
| 50 | 14.963 | 4.58 | 94 | 67.05 | 9,12-Octadecadienoic acid (Z,Z) | Fatty acid (polyunsaturated) | Reliable |
| 51 | 15.106 | 1.2 | 84 | 100.05 | Octadecanoic acid | Fatty acid (saturated) | Reliable |
| 52 | 18.178 | 1.15 | 90 | 67.05 | 9,12-Octadecadienoic acid (Z,Z), 2-hydroxy-1-(hydroxymethyl) ethyl ester | Ester (fatty acid derivative) | Reliable |
| 53 | 30.755 | 1.83 | 75 | 313.15 | rac 1-Oleoyl-2-palmitoylglycerol | Lipid (glyceride) | Tentative |

Note: Compounds with similarity index ≥80% were considered reliable identifications, while those with lower similarity values were treated as tentative assignments. Peak numbers correspond to Figure 4. Some compounds were detected at multiple retention times, possibly representing isomers or co-eluting compounds. The m/z values represent the base peak (most abundant ion) in the mass spectrum

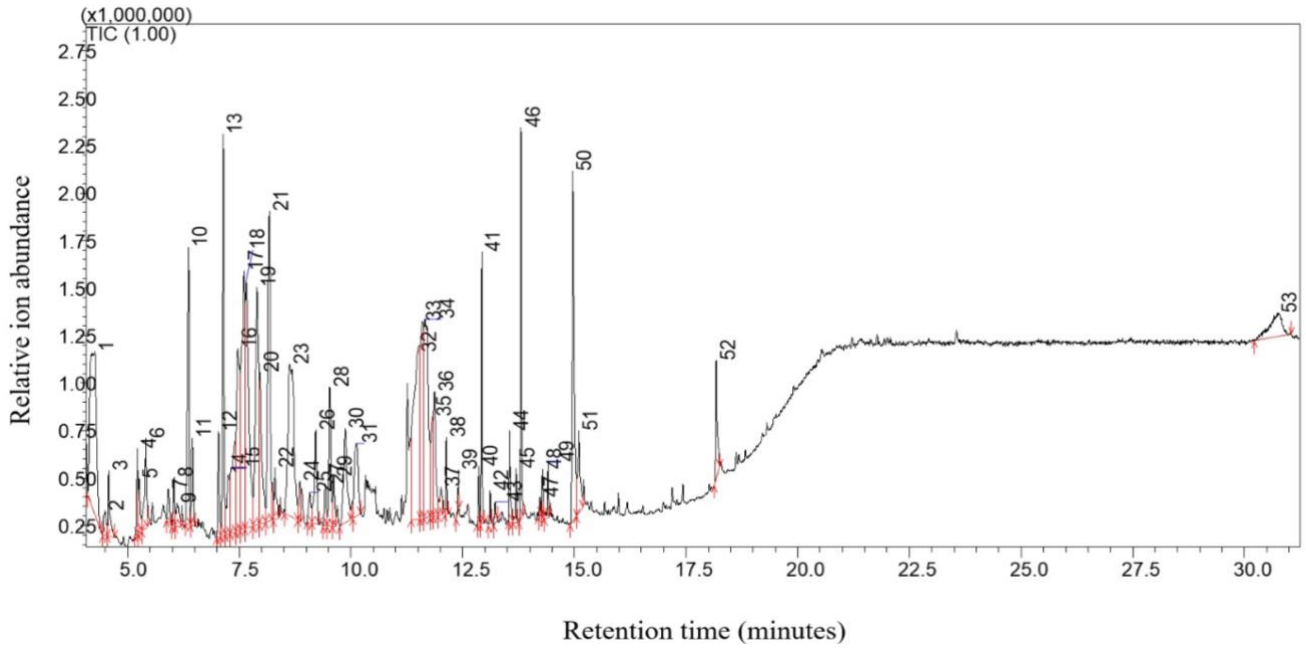


**Figure 4.** GC-MS Total Ion Chromatogram (TIC) of volatile and semi-volatile compounds detected in the ethyl acetate extract of the *Streptomyces* isolate. Peaks (1-53) are labeled and correspond to the compounds listed in Table 1 based on retention time and spectral identification

In addition, a group of detected compounds belongs to fatty acids and lipid derivatives, including tetradecanoic acid, pentadecanoic acid, n-hexadecanoic acid, palmitoleic acid, octadecanoic acid, and 9,12-octadecadienoic acid, which are characteristic components of microbial lipid metabolism and cell membrane structures. The remaining compounds consist mainly of phenolic compounds, furanone derivatives, pyranone derivatives, and other heterocyclic metabolites, which represent putative secondary metabolites produced by the *Streptomyces* isolate. Among these compounds were 1,2-cyclopentanedione, 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, 3(2H)-furanone, 4-hydroxy-5-methyl-, 2,5-dimethylfuran-3,4(2H,5H)-dione, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, (S)-5-hydroxymethyl-2[5H]-furanone, catechol, and 1,2,3-benzenetriol, which belong mainly to phenolic and oxygenated heterocyclic compounds commonly reported in microbial secondary metabolism. Collectively, the chromatographic profile provides a comprehensive metabolite fingerprint of the extract detected by GC-MS.

## Discussion

*Streptomyces* is widely recognized as a genus of actinomycetes capable of producing a wide range of bioactive secondary metabolites (van Keulen and Dyson 2014; Barka et al. 2016). Members of the *S. hygroscopicus* group, like many *S. lineages*, are well known for their extensive biosynthetic capacity, with numerous compounds reported from this lineage. This metabolic potential is largely driven by diverse Biosynthetic Gene Clusters (BGCs), which enable the production of structurally varied metabolites (Nett et al. 2009; Doroghazi and Metcalf 2013). Accordingly, metabolite profiling using GC-MS provides valuable insights into the expressed chemical diversity of the isolate and its potential ecological and functional roles.

The GC-MS chromatogram (Figure 4) revealed 53 volatile and semi-volatile compounds. Peaks (1-53) correspond to compounds identified based on retention time and spectral matching, as summarized in Table 1. The dominant constituents identified based on relative peak area included 3-deoxy-D-mannoic lactone (Peaks 32 and 34), (S)-(+)-2-

amino-3-methyl-1-butanol (Peak 1), 2-methylbutanoic anhydride, catechol, and 9,12-octadecadienoic acid (Z,Z). These compounds, together with other classes such as fatty acids, esters, alcohols, and phenolics, are consistent with the broad metabolic repertoire characteristic of the genus *Streptomyces*. This metabolic diversity is further supported by the genomic characteristics of actinobacteria, particularly members of Streptomycetaceae, which possess large linear chromosomes rich in Biosynthetic Gene Clusters (BGCs) that underpin the production of polyketides, nonribosomal peptides, terpenoids, and other specialized metabolites (Kämpfer et al. 2014; Barka et al. 2016). Comparative genomic analyses have demonstrated that a significant proportion of these BGCs remain cryptic or conditionally expressed, contributing to the remarkable chemical diversity observed even under standard cultivation conditions (Nett et al. 2009; Doroghazi and Metcalf 2013). The richness of compounds detected in the present isolate reflects the intrinsic genomic potential of *Streptomyces* to synthesize structurally diverse metabolites. Compounds with similarity indices ≥80% were considered reliable identifications, while those below this threshold were treated as tentative. To minimize potential background contamination, a medium blank control was analyzed under identical GC-MS conditions, and compounds detected in the control were excluded from further interpretation. Nevertheless, minor contributions from media components or solvent-derived compounds cannot be entirely ruled out. In addition, several fatty acids such as *n*-hexadecanoic acid, octadecanoic acid, and pentadecanoic acid were detected, reflecting key components of microbial lipid metabolism. These compounds align with previous GC-MS studies on terrestrial and marine actinomycetes (Mondal and Thomas 2022; Ifediora et al. 2023; Cunha et al. 2025). It should be noted that the present GC-MS analysis was conducted as a qualitative metabolite profiling approach based on a single isolate without biological replication; therefore, statistical or multivariate analyses were not performed.

Beyond their structural role in membrane architecture, fatty acids and their derivatives may contribute to antimicrobial activity, while phenolics such as catechol disrupt pathogen cell integrity through oxidative stress (Almuhayawi et al. 2021; Selim et al. 2021). In addition, volatile alcohols and esters reported in actinomycetes are known to function as infochemicals mediating interspecies communication and competitive interactions (Schöller et al. 2002; van der Meij et al. 2017). From an ecological perspective, the presence of these metabolites suggests that the pineapple rhizosphere isolate may participate actively in shaping microbial community structure through chemical interference or niche modulation. Other detected metabolites, including 3-deoxy-D-mannoic lactone and 2-methylbutanoic anhydride, have also been documented in microbial metabolomic investigations. Although functional assays were not conducted in the present study, the occurrence of these compound classes is consistent with the known metabolic versatility of the genus (Ifediora et al. 2023). These metabolites may function not only as antimicrobial agents but also as ecological mediators shaping rhizosphere microbial communities.

The detection of diverse alcohols, esters, phenolic compounds, and fatty acid derivatives suggests that the isolate may produce Volatile Organic Compounds (VOCs) associated with rhizosphere microbial communication. Bacterial VOCs are increasingly recognized as key mediators of plant-microbe interactions, influencing plant growth and microbial community dynamics through signaling processes such as root development stimulation, nutrient uptake enhancement, and induction of systemic resistance (Schulz-Bohm et al. 2017). In addition, volatile metabolites produced by *Streptomyces* and other plant-associated bacteria may suppress competing microorganisms or modulate rhizosphere communities through chemical interference. Although the present study did not experimentally evaluate these functions, the diversity of detected volatile and semi-volatile compounds suggests potential ecological roles in rhizosphere signaling and interaction processes. Future studies integrating metabolomic analysis with statistical, multivariate approaches, as well as plant bioassays and microbial interaction experiments, would help clarify the functional roles of these metabolites in the pineapple rhizosphere ecosystem.

From a comparative perspective, the predominance of lipid, ester, and phenolic derivatives observed in this isolate aligns with previous GC-MS-based analyses of terrestrial and marine actinomycetes, in which these compound classes frequently constitute major chromatographic peaks (Mondal and Thomas 2022; Cunha et al. 2025). GC-MS metabolite profiling has also been proposed as a chemotaxonomic and ecological tool, where volatile and semi-volatile signatures may serve as chemical fingerprints reflecting microbial adaptation within rhizosphere environments (Chakraborty et al. 2022; Ifediora et al. 2023). In this context, the metabolite diversity detected in the pineapple rhizosphere isolate suggests metabolic flexibility that may facilitate persistence in complex soil microbial communities.

Phylogenetic analysis of 16S rRNA gene sequences positioned the isolate within a clade affiliated with members of the *S. hygroscopicus* group. The scale bar (0.05) in the phylogenetic tree represents genetic distance (substitutions per site) rather than percentage similarity; therefore, phylogenetic relationships were interpreted based on clustering patterns and overall tree topology rather than direct percentage similarity values. The 16S rRNA gene remains a cornerstone for actinobacterial systematics and preliminary species delineation (Stackebrandt and Schumann 2006; Yoon et al. 2017). In this context, the isolate is conservatively referred to as affiliated with *S. hygroscopicus* in the present study. Confirmation at the species level would require additional analyses such as Multi-Locus Sequence Analysis (MLSA). The relationship between 16S rRNA similarity and genomic coherence has been discussed in the context of average nucleotide identity thresholds for species demarcation (Kim et al. 2014), highlighting the importance of integrating molecular data with phenotypic characteristics. Notably, lineages related to *S. hygroscopicus* are well documented for prolific secondary metabolite production. Genome-based investigations have revealed numerous BGCs in such strains, supporting the biosynthesis of antibiotics and other bioactive molecules

(Nett et al. 2009; van der Meij et al. 2017). The congruence between phylogenetic placement and chemical complexity observed in this study strengthens the interpretation that the isolate harbors substantial biosynthetic capacity.

From a biocontrol perspective, *S. hygroscopicus* has been extensively explored as a biological control agent against fungal and bacterial plant pathogens (Law et al. 2017; Torres-Rodríguez et al. 2022). These mechanisms encompass antibiosis, competition for nutrients and space, and induction of systemic resistance in host plants (Khan et al. 2023a). The detection of diverse secondary metabolite classes in the present isolate provides a biochemical foundation for potential antagonistic activity, although targeted bioassays and mechanism-oriented studies are required to confirm functional efficacy. The integration of metabolomic screening with phylogenetic identification, as performed here, offers a rational strategy for prioritizing promising isolates for downstream application.

However, this study is subject to several limitations. The analysis was based on a single isolate from a single sampling location, and the GC-MS profiling was conducted as a qualitative, non-replicated assessment without statistical validation or functional bioassays. In addition, species-level identification remains tentative due to the reliance on 16S rRNA gene analysis alone, and the biological activities of the detected metabolites were not experimentally confirmed. Future research should therefore incorporate Multi-Locus Sequence Analysis (MLSA) or whole-genome sequencing to achieve precise taxonomic resolution, as well as quantitative metabolomics and multivariate statistical analyses to validate metabolite consistency. Functional assays, including antimicrobial testing, plant growth promotion experiments, and rhizosphere interaction studies, are also essential to elucidate the ecological roles and biotechnological potential of the detected compounds.

In conclusion, this study provides a comprehensive polyphasic characterization of a *Streptomyces* isolate obtained from the pineapple rhizosphere in Lampung, Indonesia, integrating morphological, molecular, and metabolite-based analyses. Morphological observations using scanning electron microscopy confirmed characteristic features of the genus, including extensively branched hyphae and rectiflexibiles-type spore chains. Molecular identification using 16S rRNA gene sequencing (≈1,450 bp) placed the isolate within the *S. hygroscopicus* clade, showing low genetic divergence (<0.02 substitutions per site) relative to closely related reference strains, thereby supporting its taxonomic affiliation while indicating the need for higher-resolution genomic approaches for definitive species delineation. GC-MS metabolite profiling revealed a chemically diverse extract comprising 53 volatile and semi-volatile compounds, with spectral similarity indices ranging from 66% to 96%. Major constituents included 3-deoxy-D-mannoic lactone (6.92%), (S)-(+)-2-amino-3-methyl-1-butanol (6.42%), 2-methylbutanoic anhydride (5.74%), catechol (5.16%), and 9,12-octadecadienoic acid (Z,Z) (4.58%), representing multiple chemical classes such as organic acids, alcohols, phenolic compounds, heterocyclic derivatives, and fatty acids. This metabolite diversity reflects the intrinsic biosynthetic capacity of *Streptomyces* and suggests potential ecological roles in rhizosphere interactions, including microbial competition and chemical signaling. Overall, this study provides baseline taxonomic and metabolomic insights into pineapple-associated *Streptomyces* in Indonesian agroecosystems and establishes a foundation for future functional and applied investigations.

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